• Analyzed purity experiments of CYpet-SUMO1 and Ypet-Ubc9 at constant concentration of 1µg from 100% to 10%. Results: We ran samples in two wells and got overall similar graphs from 100% to 10%. The data and graphs suggest that fluorescence of an unbound CYpet-SUMO1 and Ypet-Ubc9 does not change significantly as purity changes.

• Analyzed FRET purity test at 500 ng per protein. Results: We ran three samples to obtain three graphs with all concentrations the same for each well. Overall the FRET ratios are similar with 40 percent purity having the highest FRET ratio. It is unknown if this holds significance at higher concentrations since 500 ng is a low quantity.

• Analyzed third sensitivity test for CYpet-SUMO1 and Ypet Ubc9. Results: experiment followed the same trend as in the first two sensitivity with the lowest registered signal was at 25 ng/ml. However the noise signals register at similar levels as the 25 ng/ml. Therefore the results confirm that 500 ng is the accurate choice for avoiding noise signals skewing data.

• Expressed 1 liter of CYpet-SUMO1 and purified using three different washing protocols similar to the first weekly report.
• Set up and ran Bradford test and Fluorescence Intensity test for fluorescence purity based on fluorescence protein concentrations to total protein concentration. Results: After analyzing both tests and calculating purity, the purification protocol that had the highest purity was protocol 3 at 42 percent. The results for all three protocols in both tests showed higher yields and better purities for CYpet-SUMO1 than the previous tests done with our last batch of expressed protein.

Next week

• Express more CYpet-SUMO1 and Ypet-Ubc9 for all tests and optimizations written below.
• Check FRET ratio at higher quantities than 500 ng to see if results differ.
• Individual fluorescence of CYpet-SUMO1 and Ypet-Ubc9 purity tests at higher quantities than 500 ng and analyze results.
• Start the optimization on the Lyophilization protocol.